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Potential synergestic effect of quercetin and exercise on depressive-like behavior in male albino rats: Targeting oxidative stress, inflammation, neural apoptosis, and mitophagy

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- BDNF
- Exercise
- Quercetin
- PINK1/Parkin

Abstract

This work was conducted to assess the effects of quercetin and exercise on depressive-like behavior. Fifty Albino rats were categorized into five groups, **Control group** (saline + vehicle) for 10 days**, Depression group**: lipopolysaccharide (LPS) was intraperitoneally (IP) injected (0.5 mg/kg/day) for 10 days, **Depression group treated by quercetin**: LPS was IP injected (0.5 mg/kg/ day) then intragastric injection of quercetin (40 mg/kg/day) for another 10 days, **Depression group treated by exercise**: LPS was IP injected (0.5 mg/kg/day) then rats was treated with treadmill exercise for another 10 days, and **Depression group treated by quercetin and exercise**: LPS was injected, then quercetin was administered in addition to treadmill exercise. At the end of the experiment, behavioural changes were examined. H_2O_2, MDA, GSH, IL-1β, TNF-α, caspase-3, and brain neurotransmitters such as serotonin and BDNF were assessed, and PINK1 and Parkin mRNA levels were assessed. A histopathological assessment of the hippocampus was performed. LPS elicited behavioral impairments and substantially augmented H_2O_2, MDA, IL-1β, TNF-α, caspase-3, PINK1 and Parkin levels, with considerably reduced serotonin, BDNF and GSH levels. Meantime**,** quercetin and exercise effectively suppressed LPS-induced cognitive impairment and attenuated H_2O_2, MDA, IL-1β, TNF-α, caspase-3, PINK1 and Parkin levels, and enhanced serotonin, BDNF and glutathione levels, with greater impact reported in the combination group. Our findings suggest that quercetin combined with treadmill exercise can relieve LPS-induced depression-related behavior via modulation of redox status, inflammation, neuronal apoptosis, and mitophagy.

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1. Introduction

It is stated that depression is a mental disorder of low mood and reluctance to activity, depressive conditions (e.g., major depressive disorders) affects a one's thoughts, behavior, feelings, and sense of wellbeing, with often loss of motivation or interest (**1**).

Depression (formerly dysthymia) is a normal temporary reaction (short or long terms) to life events. It is represented as sadness, difficulty in thinking and concentration and a marked increase or decrease in appetite and duration of sleeping, with feelings of hopelessness and suicidal tendencies (**2**).

Regarding that depression is mainly determined by cognitive defect and restlessness, which can be related to dysfunctions of hippocampus and prefrontal cerebral cortex within brain which are responsible for decision making. Also, stress and depression are intimately related, possibly via the hypothalamic pituitary adrenal (HPA) axis upon exposure to stressful stimuli (**3**).

Attitudes of depressive behavior have been detected in experimental animals, which can be reduced after antidepressant medications, as features of helplessness, anhedonia (reduced motivation), behavioral desperation, and alterations in sleep and appetite styles, which seem to be physiological rather than pathological disorders (**4**).

Most antidepressant drugs share in correcting neurotransmitter imbalances affecting mind and emotions, enhancing the monoamine system (e.g. serotonin, dopamine, and norepinephrine) transmission within cognitive brain structures, but there are several adverse effects of antidepressant drugs, such as drug resistance and a long latent period and numerous side effects (**3**). Thus, phytotherapy (medications extracted from plants) appears to be more effective and with less adverse effects (**5**).

Quercetin is an important bioactive flavonoid (secondary metabolites) extracted from several types of plants (fruits and vegetables), it has been used against free radicals (oxidants) integrated for development of related diseases, like hypertension, vascular disorders (e.g., atherosclerosis), and metabolic syndrome (including obesity) (**6**).

It has been shown that quercetin may regulate levels of neurotransmitters, improving regeneration of neurons of hippocampus as well as HPA axis interruption, with decreasing inflammatory conditions and oxidative stress (OS), theses pathological signs are present in depressive disorders (**7**).

Physical activity and exercise have various advantages, including both body and mentality. It can be proved that exercise having multiple benefits on the body from the neck down, which is useful for people suffering from major depressive disorders (**8**).

Exercise is included as a management for decreasing body weight, in addition to enhancing sleep characters (**9**). Also, exercise can act against stress induced inflammatory conditions (via effects on hypothalamic–pituitary–adrenal) (**10**), with improving both immune system and autonomic balance (**8**).

In this study, we aimed to compare the efficacy of quercetin, exercise and both together in animals with depressive like behavior.

2. Material and methods

2.1 Chemicals

Lipopolysaccharides (LPS) (Escherichia coli 0111:B4), Quercetin (CAS no: 117-39-5), and all other chemicals used from Sigma-Aldrich Chemicals (St. Louis, MO).

2.2 Animals

This study was conducted on 50 male adult albino rats of the local strain with average weight (190-230 g) collected from Tanta University Animal House. Animals were housed in standard well-ventilated wire mesh cages in standard laboratory conditions, with free access to water and food pellets during the entire experiment. The maximum number of rats per cage was restricted to three to prevent overcrowding and reduced cleanliness. Rats were monitored five times a week for signs of cage aggression. All experiments were done following the rules of the Tanta University ethical committee (code: 36264PR289/8/23).

2.3 Animal groups

After undergoing acclimatization for one week, the rats were assigned to five groups (n=10).

Group I: Control group: received intraperitoneal injection (i.p.) of 0.5 mL sodium chloride solution for 10 days, then intragastric 0.5% carboxymethyl cellulose (CMC) for another 10 days.

Group II: depression non treated group: The rats received daily i.p. LPS injection (0.5 mg/kg, dissolved in sodium chloride solution (0.9%) for 10 days **(11)**, then intragastric 0.5% carboxymethyl cellulose (CMC) for another 10 days.

Group III: depression treated group by quercetin: The rats received daily i.p. LPS injection (0.5 mg/kg, dissolved in sodium chloride solution (0.9%) for 10 days, then received 40 mg/kg intragastricquercetin (dissolved in 0.5% CMC) for another 10 days **(12).**

Group IV: depression treated group by exercise: The rats received daily i.p. LPS injection (0.5 mg/kg, dissolved in sodium chloride solution (0.9%) for 10 days, then treadmill exercise for another 10 days.

Group V: depression treated group by quercetin and exercise: The rats received daily i.p. LPS injection (0.5 mg/kg, dissolved in sodium chloride solution (0.9%) for 10 days, then received 40 mg/kg intragastric quercetin (dissolved in 0.5% CMC) along with treadmill exercise for another 10 days.

2.4 Behavioral tests

The behavioral tests were conducted at the conclusion of the experimental period, including the forced swimming test and tail suspension test.

2.4.1 Forced swimming test

The rats were required to undergo separate swimming sessions after learning how to swim in a training session. During the experiment, each rat was subjected to a 75% water-filled cylinder with a height of 50 cm and a diameter of 22 cm, which was filled with water to the extent of its total volume to prevent rodent escape. The rats were gradually introduced to the water over five minutes. For each rat, the floating, swimming, and struggling times were recorded in seconds **(13).**

2.4.2 Tail-suspension test

As part of this test, rats were subjected to a brief period of stress. This test is commonly used for examining depressive-like behavior. The rats were held 25 cm above the ground with adhesive tape around their tails. To prevent the rats from climbing or fleeing, adhesive tape was applied to the tail ends. A complete observation period of six minutes was noted for the average mobility duration. Rats were considered immobile if they

displayed complete lethargy without moving. Each rat's immobility time was recorded in seconds **(14).**

2.5 Tissue sampling.

At the end of the experimentation period, rats were anesthetized by i.p. injection of pentobarbital (50 mg/kg) **(15),** sacrificed by cervical dislocation. A complete dissection of the brain was conducted, and the brain was dissected into three fractions. The first fraction was fixed in 10% formol saline solution for 24 h and then stained with hematoxylin and eosin for histological analysis by light microscopy. The second one was homogenized in 50 mM phosphate buffer saline, pH 7.4. The tissue homogenate was centrifuged at 1,000 rpm for 10 min at 0 ^{o}C in a cold centrifuge. The supernatant was isolated and maintained at −20 ^{0}C for total protein content assessment (**16**) and biochemical assays. The final part was frozen at −80 ^{0}C for molecular analysis.

2.6 Biochemical assays

2.6.1 Assay of oxidant/antioxidant biomarkers

Hydrogen peroxide (H_2O_2) level in brain tissue was determined spectrophotometrically, which relies on H_2O_2's ability to react with 3, 5-dichloro-2 hydroxybenzensulfonic acid and 4-aminophenazone in the presence of peroxidase forming a chromophore (Quinoneimine Red Dye) (**17**). Brain tissue malondialdehyde (MDA) level was assayed spectrophotometrically by a thiobarbituric acid approach reported by (**18)** while brainglutathione (GSH) content was estimated according to (**19)** using BIODIAGNOSTIC kits (Giza. Egypt).

2.6.2 Assay of inflammatory and apoptotic markers

Brain interleukin (IL)-1β, tumor necrosis factor-α (TNF-α), and caspase-3 levels were estimated by rat-specific ELISA kits (RayBiotech Inc., GA, USA), based on the manufacturer's protocol.

2.6.3 Assay of neurotransmitter content

Rat ELISA kits (MyBioSource, San Diego, CA, USA) were utilized to measure serotonin and BDNF contents in brain tissue.

2.6.4 Quantitative Real-Time PCR Analysis

Frozen brain tissue was collected to isolate total RNA with the help of Gene JET RNA Purification Kit (Thermo Fisher Scientifc Inc., #K0731, USA). Total RNA was reverse transcribed into cDNA by RevertAid H Minus Reverse Transcriptase Kit (Cat#K1632, Thermo Fisher Scientifc) as described in the manufacturer's protocol. The cDNA was used as a template to detect PINK1 and parkin gene expression using the Step OnePlus real-time PCR system (Applied Biosystem, Life Technology). Quantification of the target mRNA levels was calculated in relation to the internal control, β-actin. The primer's sequences were: rat PINK1: sense (5′CATGGCTTTGGATGGAGAGT3′) and anti-sense (5′TGGGAGTTTGCTCTTCAAGG3′); rat parkin: sense (5′CTGGCAGTCATTCTGGACAC3′) and anti-sense (5′CTCTCCACTCATCCGGTTTG3′); rat β-Actin: sense (5-GGCTGTGTTGTCCCTGTAT-3′) and anti-sense (5- CCGCTCATTGCCGATAGTG-3′). Relative gene expression was determined using the threshold cycle $2^{-\Delta\Delta Ct}$ method.

2.7 Statistical analysis

All data was displayed using the mean ± SD. **One-way ANOVA** and **Tukey's** post-hoc test were applied to analyze and evaluate significance. A P-value less than 0.05 was considered statistically significant. **SPSS software** (Version 23.0) was employed for statistical analyses.

3. Results

3.1. Effect of quercetin and exercise on behavioral tests for depressive-like behavior in Albino rats.

As illustrated in **Figure 1**, LPS injection markedly shortened the duration of struggling and swimming time and significantly prolonged time spent floating in the LPS-induced depressive rats compared with controls. Interestingly, both quercetin and exercise exerted an anti-depressant effect, as evidenced by effectively increasing the duration of struggling and swimming time and declining time spent floating, with better results recorded in the combined treated group (Group V). In the tail suspension test, the LPS group's immobility time was significantly longer than the control group. Meanwhile, treatment with quercetin and exercise substantially decreased immobility time in all treated groups compared to the untreated depression one **(Figure 1).**

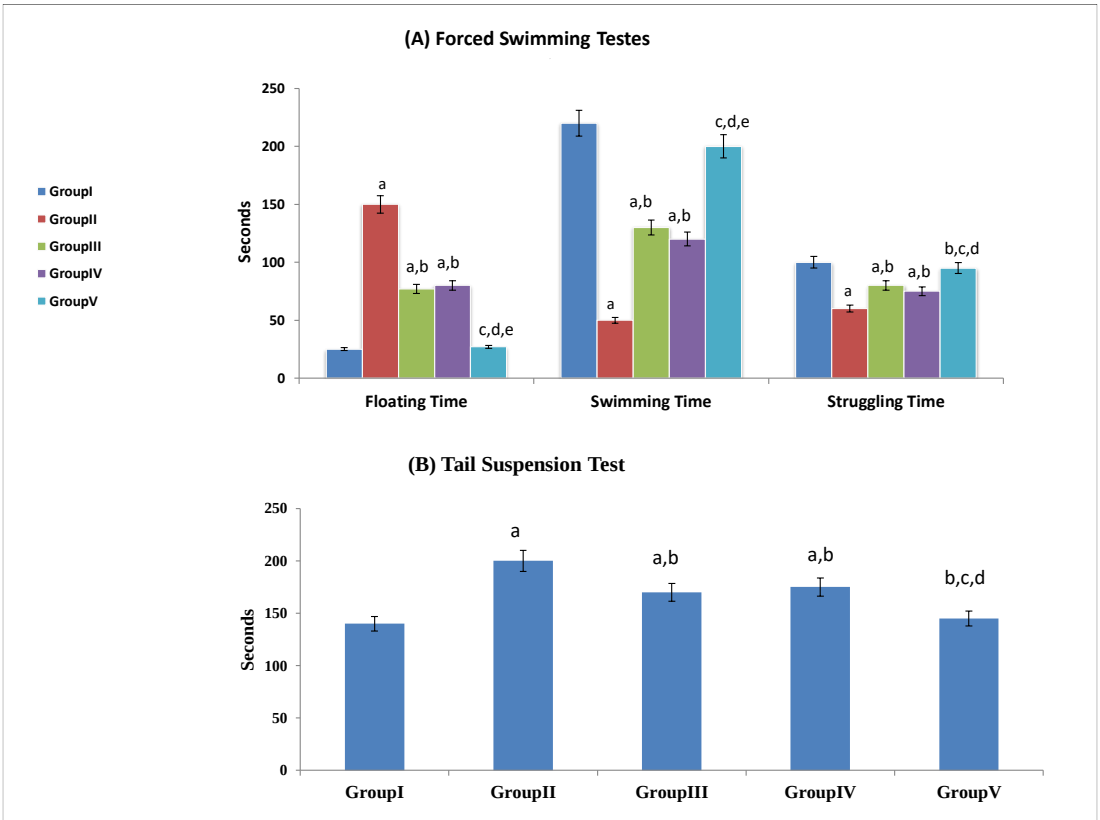

Figure (1) Effect of quercetin and exercise on behavioral tests.

[a], [b], [c] and [d] denote statistical significance at $p \leq 0.05$, [a] Vs group I, [b] Vs group II, [c] Vs group III, [d] Vs group IV.

3.2. Effect of quercetin and exercise on oxido-inflammatory and apoptotic markers in depressive-like behavior in Albino rats.

Table (1) reveals a profound escalation in brain H_2O_2, MDA, TNF-α, IL-1β, and caspase-3 levels, along with a marked reduction in GSH content in brain tissue in the depressive group compared to the control group. Meanwhile, the treated groups effectively reversed the above-mentioned parameters with superior improvement displayed in the combined quercetin and exercise-treated group.

Table (1) Effect of quercetin and exercise on oxido-inflammatory and apoptotic markers in depressive-like behavior.

	Group I	Group II	Group III	Group IV	Group V
Hydrogen peroxide (nmol/g tissue)	1.43±0.07	3.47±0.31 [a]	2.33±0.36 [a,b]	2.47±0.39 [a,b]	1.45±0.08 [b,c,d]
MDA level (nmol/g tissue)	2.34±0.15	4.17±0.32 [a]	3.2±0.02 [a,b]	3.7±0.03 [a,b]	2.37±0.09 [b,c,d]
TNF-α (pg/mg pt)	16.77±2.55	33.55±4.17 [a]	24.87±2.67 [a,b]	26.93±2.68 [a,b]	18.67±2.25 [b,c,d]
IL1β (pg/mg pt)	45.1±5.79	103.15±9.53 [a]	67.67±7.33 [a,b]	70.23±8.46 [a,b]	49.09±3.89 [b,c,d]
Caspase 3 level (ng/mg pt)	0.69±0.16	5.43±1.36 [a]	2.87±0.78 **a,b**	3.47±0.94 [a,b]	0.90±0.23 [b,c,d]
GSH level (umol/mg pt)	0.2±0.058	0.07±0.028 [a]	0.14±0.06 [a,b]	0.13±0.04 [a,b]	0.18±0.04 [b,c,d]

[a], [b], [c] and [d] denote statistical significance at $p \leq 0.05$, [a] Vs group I, [b] Vs group II, [c] Vs group III, [d] Vs group IV.

3.3. Effect of quercetin and treadmill exercise on neurotransmitter content in depressive-like behavior.

LPS-injected rats demonstrated a marked reduction in serotonin and BDNF in neuronal tissue relative to those of control rats. In contrast, the treated groups (Groups III, IV, and V) showed substantial escalation in serotonin and BDNF levels compared to the depression group **(Figure 2).**

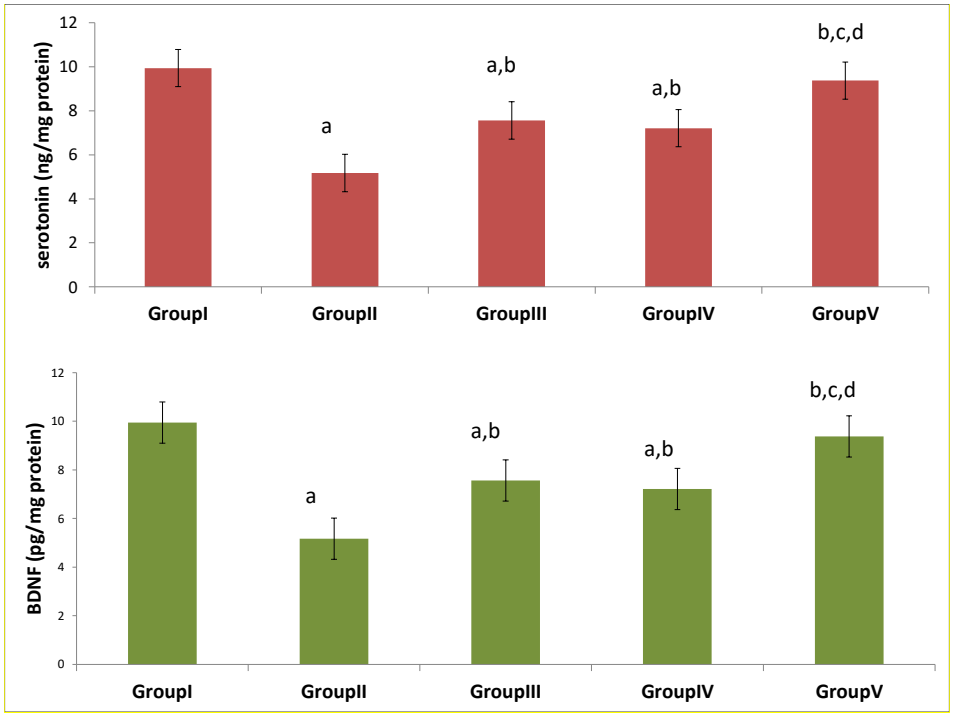

Figure (2) Effect of quercetin and exercise on serotonin (ng/mg protein) and BDNF (pg/mg protein)in depressive like behavior

[a], [b], [c] and [d] denote statistical significance at $p \leq 0.05$, [a] Vs group I, [b] Vs group II, [c] Vs group III, [d] Vs group IV.

3.4. Effect of quercetin and exercise on PINK1 and Parkin mRNA levels in depressive-like behavior in Albino rats.

As depicted in **Figure 3,** PINK1 and Parkin mRNA relative expressions in brain lysates were markedly upregulated in the LPS group than in the Control rat. However, they were significantly downregulated after quercetin and exercise treatment, with better efficacy reported in the combined quercetin and exercise-treated group.

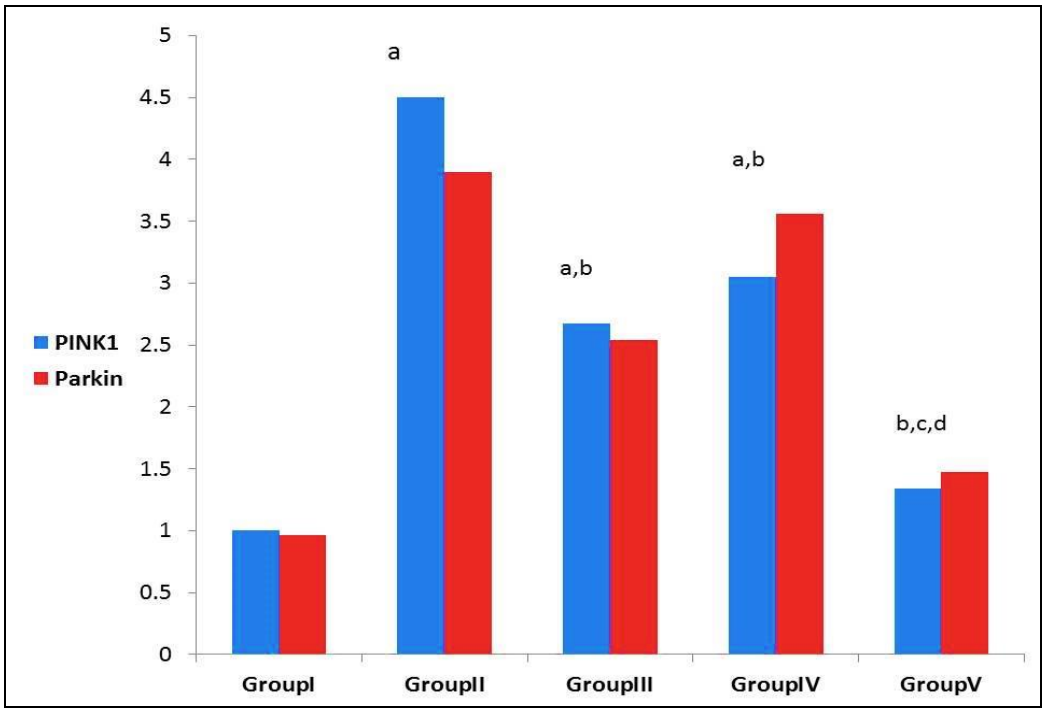

Figure (3) Effect of quercetin and exercise on PINK1 and Parkin mRNA levels.

[a], [b], [c] and **[d]** denote statistical significance at $p \leq 0.05$**, [a]** Vs group I, **[b]** Vs group II, **[c]** Vs group III**, [d]** Vs group IV.

3.5. Effect of quercetin and exercise on histological structure of hippocampus in depressive-like behavior.

Assessment of H&E stained sections revealed that the stratum pyramidale of CA1 of the hippocampus in the control group contained 2-3 layers of small pyramidal cells with vesicular nuclei, prominent nucleoli and apical dendrites. The CA1 region in the depressed rats displayed disturbed arrangement of small shrunken pyramidal cells with pyknotic nuclei. The apical processes of the cells were lost**.** In group 3, CA1 region showed pyramidal cells with vesicular nuclei and few cells with pyknotic nuclei were hardly identified. Some apical dendrites appeared. In group 4, the CA1 region showed pyramidal cells with vesicular nuclei but some cells with pyknotic nuclei still present. Apical dendrites couldn't be identified. In group 5, CA1 retained an apparently normal architecture of stratum pyramidale with small pyramidal cells having rounded vesicular nuclei and well-formed apical dendrites **(Figure 4).**

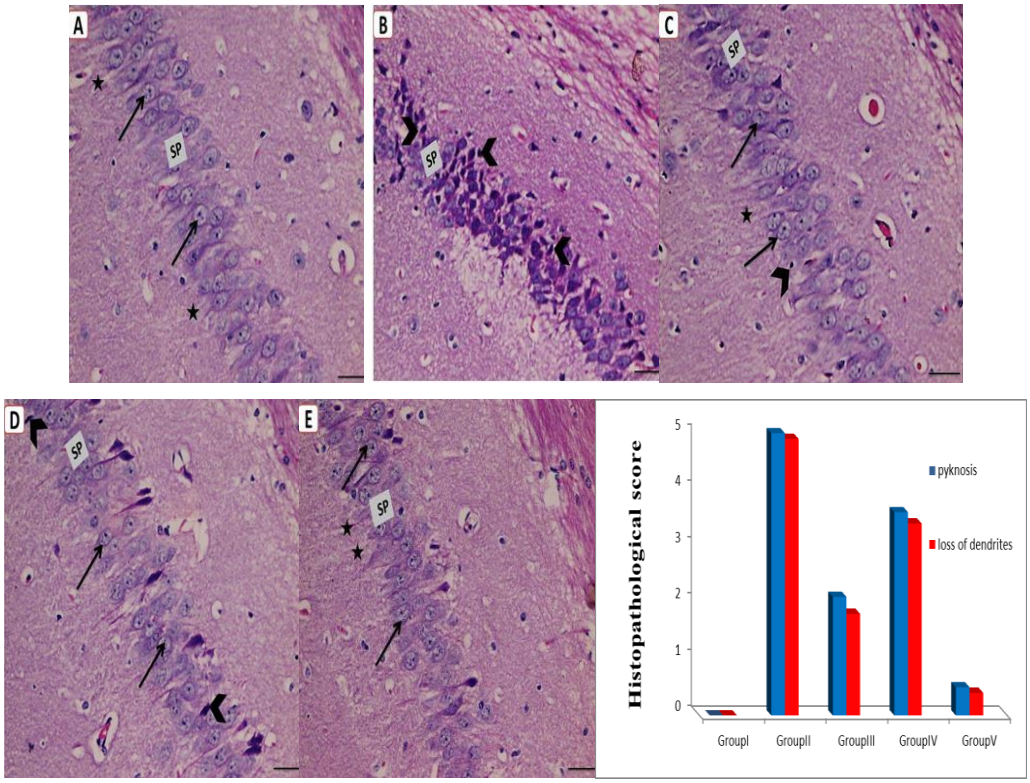

Figure (4) Effect of quercetin and exercise on histological structure of hippocampus

A: (stratum pyramidale (SP) of control group showed pyramidal cells with vesicular nuclei and prominent nucleoli (black arrows). Apical dendrites are well identified (stars). **B**: Sp of depression group showed disturbedshrunken pyramidal cells with piknotic nuclei (arrow heads). Notice: The dendrites are lost. **C**: Sp of quercetin treated group showed pyramidal cells with vesicular nuclei (black arrows). Few cells with pyknotic nuclei (arrow head) and some apical dendrites appear (star). **D**: Sp of exercise treated group showed pyramidal cells with vesicular nuclei (black arrows) and some cells with piknotic nuclei (arrow heads). Apical dendrites can't be identified. **E**: Sp of group 5 showed restoration of the pyramidal cells that arrangement with vesicular nuclei (black arrows). Apical dendrites of the cells are present (stars). (X: 400, bar 50 µm). Histopathological score (piknosis & loss of dendrites).

4. Discussion

The current work aims to discuss the effects of quercetin and exercise on depressive like behavior (produced by lipopolysaccharide) in male albino rats. Novel interventions for alleviating depression are still of considerable interest. Although the precise etiopathogenic mechanisms remain uncertain, emerging evidence indicates that LPS activate the microglial system and stimulate a cascade of inflammatory reactions to induce depression.

Cavaillon., 2018, suggested that the mechanism of depression induced by LPS which activates inflammatory cells as monocytes (later macrophages) and endothelial cells, to stimulate cellular signaling systems, increasing several cytokines and inflammatory biomolecules levels (**20**). These inflammatory signals are transmitted to the CNS through endothelial cells (a part of blood brain barrier), leading to brain neuroinflammation which induce depression (**21**). **Ji et al., 2020,** stated that LPS is accused of interruption of function of a parvalbumin interneuron (gamma

aminobutyric acid releasing cortical interneurons), the previous mechanism mediates generalized inflammation–induced depression in the form of abnormal behaviours and reduction in exercise capacity **(22)**.The present work demonstrated considerableincreases in immobility time of both forced swimming test (FST) and tail suspension test (TST) in LPS induced depression, in the form of increased floating time (only raising heads) and decreased swimming time (decreased resistance) in FST, and decreased struggling time in TST, which were significantly reversed after quercetin treatment or exercise, and were more significantly reversed (near or above normal) after both treatment and exercise.

Quercetin treatment significantly improved stress modulated behavior disturbance, which is evidenced by improved locomotion, lesser immobility time and greater frequency of upward turning in TST and FST **(23).**The quercetin antidepressant action is reported to be via inhibiting the enhanced hypothalamic pituitary adrenal (HPA) axis in depressed mice (evidenced by decreased immobility time in FST), with the decreasing of cortisol levels. Also, quercetin was stated as a potent agent as diazepam in decreasing adrenocorticotropic hormone (ACTH) and cortisol levels in mild traumatic brain injury **(7).**

According to **Jung et al., 2023**, resistance training (3 times/ week) in mice for 4 weeks, prevented anxiety/depression-like symptoms, neuroinflammation, inflammasome activation (inflammatory cellular proteins), and suppressed the BDNF/ receptor tropomyosin receptor kinase B (BDNF/ TRkB) signaling pathway in the hippocampus **(24)**. BDNF/ TRkB signaling pathway in turn phosphorylates the protein kinase B/ mammalian target of rapamycin (Akt/mTOR) pathway responsible for the expression of proteins integrated for neuronal proliferation or regeneration, which markedly shortens the immobility time in FST and TST **(24).**Also **Henrique et al., 2018**, reported that exercise reduce inflammatory markers in the cerebral cortex and hippocampus of rats exposed to chronic stress (**25**).

Our work demonstrated a notable increase in malondialdehyde (MDA), TNF-α and IL-1β in LPS-induced depression, indicating neuroinflammation, which were significantly decreased after quercetin treatment or exercise, and were more significantly decreased (near normal) after combined treatment. We reported significant decrease in GSH in LPS-induced depression (decline in antioxidant capacity), which were significantly increased after quercetin treatment or exercise, and were more significantly increased (near normal) after combined treatment. **Choudhary et al., 2023,** reported superoxide dismutase (SOD) enzyme acts as an antioxidant against OSgenerated by reactive oxygen species (ROS), SOD catalyzed the conversion of free radicals (O^{-2}) into stable molecular oxygen and H_2O_2.Also, reduced glutathione (GSH) is necessary for redox balance against free radicals as H_2O_2 (forming oxidized glutathione GSSG and H_2O) by the action of glutathione peroxidase (GPx). Reduced glutathione level was declined after LPS administration (which increased ROS), with increased MDA level (lipid peroxidation by ROS). As well as the increased IL-1β the known as the first cytokine to enhance HPA axis during the immunological response occurring within depressive disorders **(26).** Increased formation of

ROS activates HPA axis. So, the increased glucocorticoids activate cellular glucocorticoid receptors (GRs) to increase the mitochondrial membrane potential, calcium holding capacity, and mitochondrial oxidation, leading to production of H_2O_2 and hydroxyl radicals (OH^-) causing oxidative cell damage **(27)**. Also, LPS caused a marked increase in hemeoxygenase (HO) activity, especially in hippocampus, which was associated with increased levels of nitric oxide (NO) activity, showing brain OS indicated by regional distribution of lipid peroxides (for MDA production), that may lead to permanent dysfunction or cell apoptosis **(28).**

Quercetinand exercise treatment improved the inflammatory and oxidative states in the form of significantly decreasing oxido inflammatory markers (MDA, TNF-α, IL-1β and hydrogen peroxide levels in the hippocampus while significantly increase anti antioxidant marker GSH, better results were observed in combined treatment. Quercetin inhibits lipid peroxidation (reducing MDA) mainly by acting as a scavenger against free reactive radical, and strong inducer of detoxifying enzymes as SOD, catalase (decreasing H_2O_2) and GPx **(29).** Also, according to **Chen et al., 2022,** quercetin derivative markedly relieved mouse depression-like behaviors, via suppression of the mitogen-activated protein kinase kinase/ extracellular signal-regulated kinase/ nuclear factor-κB (MEK/ERK/NF-κB) axis in hippocampus (switching off the phosphorylating enzymes of cell division), decreasing IL-1β, IL-6, TNF-α levels **(7)**. Regular exercise acts as an anti-inflammatory in depressive disorders. MDA levels were diminished with continuous exercise (for 150 minutes per week), as well as TNF-α & IL-6 levels were declined (without significant changes in IL-1β). This is explained by the anti-inflammatory role of continuous exercise against neutrophil infiltration, lipid peroxidation, and pro-inflammatory cytokine responses. This is in addition to the increased mitochondrial biosynthesis of antioxidant enzymes. SOD and GPx activity (restoration of GSH) **(30)**.

Our work showed a significant increase in brain caspase-3 level in LPS-induced depression indicating neuronal apoptosis, which were significantly decreased after quercetintreatment or exercise, and were more significantly decreased (near normal) after combined treatment.LPS administration into rats induces microglial activation and production of proinflammatory cytokines in the hippocampus exhibiting neuroinflammation and depression-like behaviors, this is associated with mitochondrial and cell autolysis. Several cytokines activate NF-κB and protein 38 (p38) mitogen-activated protein kinase (MAPK) signaling pathways, to induce neuronal apoptosis by up regulating apoptosis proteins caspase family **(31)**. Administration of quercetin markedly lowered caspase-3 activity, by increasing antiapoptotic proteins and lessened both proapoptotic proteins (Bax) and cleaved caspase-3, reducing neuroinflammation and apoptosis in hippocampus **(3)**. Regular exercise decline level of proapoptotic proteins and increase brain derived trophic factor (BDNF) levels, both causing downregulation of the active phosphorylated-Janus kinase/ Janus kinase (p-JNK/ JNK) ratio, subsequently inhibiting caspase-3 cleavage and enhancing Bcl2 expression **(32)**.

Our work showed a significant decrease in brain neurotransmitters as serotonin and BDNF in

LPS-induced depression, which were significantly increased after quercetin treatment or exercise, and were more significantly increased (near normal) after combined treatment. BDNF activates the TrkB in the hippocampus and prefrontal cortex. The alternation of BDNF/TrkB signaling pathway has reduced the protein expressions of BDNFin LPS-induced depressive rats **(33)**. Quercetin would reverse these changes via modulating cytokines production and inhibiting OS and regulate BDNF-mediated disturbances of critical proteins expression implicated in neuroinflammation and neuroplasticity **(12)**. Exercise induces BDNF expression through the induction of hippocampal expression of Fndc5 (a PGC-1α and ERRα -dependent myokine). Once BDNF protein levels increase, TrkB signaling in turn inhibits *Fndc5* expression in a negative feedback mechanism **(34).**

Serotonin (5-hydroxy tryptamine) is a neurotransmitter with a crucial involvement in several neuronal functions, including sleep, stress response, cognitive function, sensory-motor and emotional regulation, via a bidirectional system (a local feedback loop) as serotonin upregulates BDNF mRNA and, in turn, BDNF–TrkB modulates the serotonergic neuronal system **(35).** LPS activates proinflammatory cytokines, to induce anhedonia (lost pleasure) in rats, which is absent after treatment with selective serotonin reuptake inhibitors (SSRIs) through inhibition of SERT (serotonin reuptake transporter) **(36)**. Also, LPS increased extracellular serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) levels in the nucleus accumbens (NAc) in basal forebrain (the neural link between motivation and action) and medial prefrontal cortex (mPFC) (**36).** Quercetin pass the blood brain barrier, causing various effects on the CNS and could be ligands for benzodiazepine binding sites of the gamma aminobutyric acid-A (GABAA) receptor that modulate the activity of other neurotransmitters (adenosine, serotonin, glycine and acetylcholine) receptors **(29).**The mechanisms of the modulation of serotonergic system after acute exercise are still unclear. The intensity of the stress condition during exercise may activate serotonergic neurons, mainly the central serotonergic neurons which can be controlled by stress related hormones as corticotropin releasing factor (CRF) and glucocorticoids **(37)**.

Our work showed a significant increase in Pink1 and Parkin gene expressions in LPS-induced depression, indicating mitophagy (autophagy of damaged mitochondria), which were significantly decreased after quercetin treatment or exercise, and were more significantly decreased (near normal) after combined treatment. LPS could induce extensive mitochondrial cleavage (and later damage) in neurons that prevents oxidative phosphorylation and ATP generation, with excessive NAD+ exhaustion and sirtuin-3 abnormalities leading to memory loss and synaptic dysfunction. All these could induce PINK1/Parkin-mediated mitophagy **(38)**. Quercetin keeps the mitochondrial structure, function and mitochondrial biogenesis in neurons, the mitochondrial biogenesis also increases oxidative phosphorylation (by inducing transcription, translation and replication), it may be due to mitochondrial response to the peroxisome proliferator-activated receptor-gamma coactivator-alpha signaling pathway, a neuroprotective pathway **(39).** Regular aerobic exercise regulates

the balance of apoptosis and autophagy (proteostasis) in the striatum, relieving the neurodegenerative pathology. Protein deglycase mutations in frontal cortex can compensate for PINK1 function to inhibit mitophagy in relation to regular exercise **(40).**Also, regular training is reported to restrict the loss of dopaminergic neurons and the increased expressions of translocases of outer membranes (TOM40 and TOM20) and translocases of inner membranes (TIM23), and the reduction of α- synuclein (which increased the expression of mitochondrial import proteins, improving mitochondrial function) **(40).**

5. Conclusion:

Depression caused by a LPS brain injury rat model, was characterized chronic inflammation, oxidative stress and disturbed neuroplasticity, which can be attenuated by quercetin treatment or regular exercise. The noticed synergistic effect between quercetin and exercise in our result promise anew strategy in treating depression.

6. Declarations and statements

6.1. Ethics approval:

Our study protocol was formulated in accordance with the recommendations of the Local Committee of Research and Medical Ethics of Tanta University's Faculty of Medicine.

6.2. Consent to publication:

Not Applicable.

6.3. Availability of data and material:

Upon request, data will be provided.

6.4. Competing interests:

The authors declare to have no conflicts of interest.

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Authors' contributions:

This manuscript was drafted, revised, and approved by all authors. They all contributed to data analysis and interpretation.

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